



Effects of alfalfa germplasm and stage of maturity on digestive process and productive response of dairy cows fed alfalfa hay-based diets

Giulio Cozzi, Martina Dorigo, Flaviana Gottardo, Paolo Berzaghi, Igino Andrighetto

Dipartimento di Scienze Animali. Università di Padova, Italy

Corresponding author: Prof. Giulio Cozzi. Dipartimento di Scienze Animali. Università degli Studi di Padova. Viale dell'Università 16, 35020 Legnaro (PD), Italy – Tel. +39 049 8272662 – Fax: +39 049 8272669
Email: giulio.cozzi@unipd.it

Paper received August 26, 2004; accepted December 27, 2004

ABSTRACT

The effects on the digestive process and the productive performances of dairy cows fed diets containing alfalfa hay from different germplasms and maturity were assessed in the present study. Three different lots of first-cut alfalfa hay were used in the study: the improved variety "Boreal", harvested at two consecutive maturity stages (early flowering and full flowering) and the ecotype "Vogherese", at full flowering. Cutting the plant at an earlier stage of maturity improved hay quality in comparison with the more mature forages (crude protein: 21.4 vs 16.5% DM; $P < 0.01$; NDF: 42.2 vs 53.5% DM; $P < 0.01$). The comparison between improved variety and ecotype carried out at the same maturity showed a higher lignin content for the latter (8.6 vs 8.2% DM; $P < 0.01$). Three isocaloric, isonitrogenous and isofibrous diets for lactating cows were formulated using an equal amount of corn silage and the maximum inclusion of one of the tested hays as forage base. The better quality of the early cut hay made it possible to increase its inclusion in the diet up to 44% of total dietary DM, while the two more mature hays covered only 36% of total DM of the respective diets. According to a 3×3 Latin square design, the diets were fed to 3 Italian Brown cows (initial average days in milk 121 ± 24 and milk yield $20 \text{ kg} \pm 1.3$) fitted with ruminal cannula in 3 consecutive periods of 28 d each. Alfalfa stage of maturity and germplasm did not affect dietary DM intake (average 16 kg/d). Degradability parameters of dietary DM, calculated by *in situ* nylon bags technique, showed similar kinetics of rumen disappearance for all diets. No differences were noticed in the ruminal rate of passage of the solid phase among diets, while the liquid phase showed a slower rate of passage for the early flowering hay diet. Consistent with the degradation process, the stage of maturity or the alfalfa germplasm did not affect the rumen fluid data or the *in vivo* digestibility coefficients of the diets. Milk yield did not show any change due to either alfalfa maturity or cultivar, while milk protein was lowered when cows received the early cut hay diet. This result was likely due to an excess of rumen degradable dietary protein which could have been limited by replacing part of the dietary protein sources (soybean meal) with others more resistant to the ruminal degradation.

Key Words: Alfalfa hay, Stage of maturity, Germplasm, Dairy feeding, Milk production.

RIASSUNTO

EFFETTO DEL GERMOPLASMA E DELLO STADIO DI MATURAZIONE DELLA MEDICA SUL PROCESSO DIGESTIVO E SULLA RISPOSTA PRODUTTIVA DI VACCHE DA LATTE ALIMENTATE CON DIETE A BASE DI Fieno DELLA LEGUMINOSA

L'obiettivo della ricerca è stato quello di valutare gli effetti della somministrazione di diete contenenti fieni di medica di diverso germoplasma e stadio di maturazione sui processi digestivi e sulle prestazioni produttive di bovine da latte. Lo studio ha considerato tre diversi fieni di medica prodotti al primo sfalcio primaverile della foraggera. Due fieni sono stati ottenuti dalla varietà migliorata "Boreal" sfalciati rispettivamente agli stadi di inizio e di piena fioritura, mentre il terzo

è stato prodotto dall'ecotipo "Vogherese" sfalciato in piena fioritura. L'anticipo dello sfalcio ha migliorato la qualità del fieno che rispetto ai prodotti più maturi ha visto un aumento del contenuto di proteina (21.4 vs 16.5% SS; $P < 0.01$) e una diminuzione della fibra (NDF: 42.2 vs 53.5% SS; $P < 0.01$). Il confronto tra la varietà migliorata e l'ecotipo della leguminosa, realizzato a parità di stadio vegetativo, ha registrato un'unica differenza di composizione chimica a carico della lignina, risultata più elevata nell'ecotipo (8.6 vs 8.2% SS; $P < 0.01$), probabilmente a causa del gigantismo che caratterizza la sua pianta. Sono state formulate 3 diete isoenergetiche, isoproteiche e isofibrose per vacche da latte ciascuna contenente una modesta quota di silomais e la massima quantità di uno dei fieni oggetto dello studio. La migliore qualità del fieno ottenuto con lo sfalcio ad inizio fioritura ha consentito di aumentare la sua quota di inclusione fino al 44% della sostanza secca (SS) totale della dieta mentre, per i 2 fieni ottenuti in piena fioritura, essa si è dovuta fermare al 36% della SS totale. Le diete sperimentali sono state somministrate a 3 vacche Brune dotate di cannula ruminale secondo un disegno sperimentale a quadrato latino 3×3 con periodi della durata di 28 d. Lo stadio di maturazione e il germoplasma della medica non hanno modificato l'ingestione di SS delle diete, risultata in media pari a 16 kg/d. La degradabilità ruminale in situ della SS delle diete non ha evidenziato differenze in funzione del tipo di medica e un simile andamento ha caratterizzato anche la velocità di transito ruminale della frazione solida. La dieta contenente il fieno prodotto all'inizio della fioritura ha invece fatto osservare un più lento transito ruminale della frazione liquida. I parametri del liquido ruminale e i coefficienti di digeribilità in vivo dei principali costituenti chimici delle diete non hanno risentito di effetti dovuti allo stadio vegetativo o al germoplasma del fieno di medica. Il tipo di medica presente nella razione non ha modificato neppure la produzione di latte che è stata in media pari a 19.6 kg/d. Il contenuto di proteina del latte ha invece risentito di un effetto dovuto alla diversa maturità del foraggio inserito nella dieta, diminuendo quando le bovine assumevano la razione formulata con il fieno ottenuto più precocemente. Il risultato è stato la probabile conseguenza di un eccesso di proteina degradabile in questa dieta ed avrebbe potuto essere corretto sostituendo parte della fonte proteica utilizzata (farina di soia) con una alimento proteico più resistente all'attacco microbico ruminale.

Parole chiave: Fieno di medica, Stadio di maturazione, Germoplasma, Alimentazione vacca da latte, Produzione di latte.

Introduction

In recent years, legume forages have been considered with great attention in diet formulation for high producing dairy cows because of their high protein content and the nutritional properties of the fibrous constituents (Hermann *et al.*, 2002; Beauchemin *et al.*, 2003). In a recent study cows fed alfalfa silage in comparison to maize silages as forage base showed a higher DM intake and a consequent increase in milk fat and protein yield because of the favourable forage effects on either ruminal fermentation pattern or on the digestibility of the fibrous constituents (Ruppert *et al.*, 2003).

These positive nutritional traits of alfalfa as a forage source for dairy cattle justify the development of genetic improvement programs by plant breeders. Nowadays, the genetic selection of the crop has made available new germplasm in addition to the local ecotypes. Many of these synthetic cultivars differ from the local ones in agronomic traits such as yield and pests resistance (Hays and Skinner, 2001). However, an ultimate decision on the use of an improved variety in dairy feeding should also take into account the nutritional characteristics of the forage and particularly its chemical composition, the digestive process (Ziliotto *et*

al., 1993; Andrighetto *et al.*, 1995) and the effects on milk production.

The aim of the present study was to compare alfalfa hay obtained from different germplasm and stages of maturity as forage base for dairy cow diets. The research protocol focused its attention on the effects of the type of forage on diet formulation, digestive process and milk response of dairy cows.

Material and methods

Alfalfa cultivars and experimental diets

The study evaluated two alfalfa germplasm, an improved variety and an ecotype. The improved variety "Boreal" comes from North America and has been registered in Italy since 1988 (Ministry of Agricultural and Forestry Policies, 2004). This cultivar is well adapted to colder climates but it can be satisfactorily grown under the environmental conditions of the Po Valley. The variety has a late autumn dormancy with good survival in winter climates and a high resistance to *Fusarium* bacteria and *Vorticillium* wilt, aphids, stem nematodes and *Phytophthora* root rot. The ecotype considered in the research is "Vogherese" a germplasm widely found in the Po Valley. Its plant is known for high development (gigantism), with

coarse stems and big leaves. It has a good survival in the Italian winter climate and a low susceptibility to *Sclerotinia trifolium*.

Three lots of first-cut alfalfa hay were used in the study; in particular the improved variety was harvested at two consecutive stages of maturity: early flowering (10% bloom) and full flowering, while the ecotype was harvested at full flowering. Four samples were randomly taken from each hay lot for subsequent chemical analysis once they arrived at the experimental station of the Dipartimento di Scienze Zootecniche (Department of Zootechnics) located within the "Lucio Toniolo" Experimental Centre of the University of Padova,

On the basis of hays composition, three experimental diets were formulated, each containing one of the tested hays. The diets were isocaloric, isonitrogenous and isofibrous and they were formulated according to NRC (2001) in order to meet the nutrient requirements of Italian Brown cows with an average milk production of 20 kg/d. The forage base of all the diets was represented by an equal amount of corn silage (19.6% of total DM) along with the maximum inclusion of a given hay. Soybean meal, dried beet pulps, maize meal and a mineral-vitamin mix were the other feed ingredients considered in the formulation process.

Animals and experimental design

According to a 3 x 3 Latin square design, three Italian Brown cows fitted with ruminal cannula were used in the study. At the beginning of the research, the cows had an average live weight of 551.0 ± 39.2 kg and a milk yield of 20 ± 1.3 kg/d and they were beyond the third month of lactation (average days in milk 121 ± 24). The experimental design considered 3 consecutive periods of 28 days each. The first two weeks of each period were considered as an adaptation phase of the animals to the given diet, while all the experimental measurements were taken in the last two weeks. The cows were housed in tie stalls and individually fed once a day at 09.00 h. The diets were offered as a total mixed ration and the ad libitum feeding was obtained by recovering after 24 h bunk residues not less than 5% of the total given. The DM intake was measured daily during the last 2 weeks of each period. Cows were weighed at the beginning and at the end of each period of the Latin square

to calculate the average live weight and the DM intake was also expressed as percentage of this value. Milk yield of the cows was measured during the same days and individual milk samples were collected at the two milkings on days 17 and 24 for composition analysis.

In situ degradability of the diets

The kinetics of DM degradability of the three diets was assessed by using the nylon bags technique of Ørskov *et al.* (1980). A representative sample (5 kg) of each diet was oven-dried at 60°C and then ground with a mill using an 8-mm screen in order to simulate the size of the dietary particles in the rumen after cow's intake (Cozzi *et al.*, 1999). A sample quantity of 4.0 ± 0.4 g was put into nylon bags 10 x 15 cm (40 µm-average pore size) to obtain a sample size/bag surface ratio of 13.3 mg/cm^2 . In each Latin square period, two bags of a given diet were incubated for 2, 4, 8, 12, 18, 24, 48, 72 and 96 h in the rumen of the cow fed the same diet. This set of incubation times was carried out from day 18 to day 26 of each experimental period. The bags were suspended in the rumen using 50 cm Teflon® bars anchored by a nylon cord at the cap of the cannula.

All incubations started at 09.00 h before the delivery of the diet to maintain a constant relationship between the initiation of the each time of incubation and the consumption of the feed by the cows. At the end of each incubation time, the bags were immediately washed to stop any residual degradative microbial activity and to remove ruminal particles from their external surface. The same washing procedure was carried out on two bags for each experimental diet to measure washing losses (time 0). After washing, the bags were oven-dried at 60°C and then weighed to measure the undegraded amount of dietary DM.

The DM degradability values obtained for each diet at different times of incubation were used to estimate the parameters of potential degradability by adopting the single component equation with lag phase proposed by Mertens and Loften (1980). The fitting procedure was computed using the derivative-free iterative method (DUD) within the non-linear regression procedure (PROC NLIN) of SAS (1989). Effective DM degradability of the experimental diets was calculated considering as

ruminal turnover the passage rate of dietary solid particles experimentally measured.

Ruminal rate of passage

The measurement of the dietary passage rate in the rumen was carried out using $\text{Na}_2\text{Cr}_2\text{O}_7$ -mordanted hays and Co-EDTA as markers for the solid and liquid phase, respectively. The marker for solid particles was prepared following the methodology proposed by Ramanzin *et al.* (1991). Samples of the three alfalfa hays were ground using a mill (2-mm screen) and then placed into 45 x 28 cm nylon bags (40 μm -average pore size). Each bag was filled with 400 g sample and it was subsequently washed with water at 90°C in order to remove all the soluble particles. After washing, the forage was oven-dried at 100°C for 5 h and then mixed with $\text{Na}_2\text{Cr}_2\text{O}_7$ (71.2 g/kg DM of forage) previously dissolved in warm water. The mixture was then oven-cooked at 100°C for 24 h. The cooked alfalfa samples were placed into polyester bags and rinsed for 3.5 h with tap water at ambient temperature. In order to remove incompletely attached Cr, the samples were left overnight in a solution of ascorbic acid with a pH < 4.5, rinsed again for 1 h with water and finally oven-dried at 100°C for 12 hours.

The Co-EDTA was obtained by mixing, 25 g of Co-acetate $4\text{H}_2\text{O}$, 29.2 g of EDTA and 4 g of NaOH into 200 ml of distilled water. The obtained product was added with 20 ml of H_2O_2 30% and it was cooled overnight. The next day, 300 ml of ethanol (95% p/v) were added and the mixture was then refrigerated for a night. The following day the liquid marker was filtered, washed with ethanol (80% w/v) and oven-dried at 60°C.

The two markers were administered in a single dose of 150 g for the mordanted hays and 35 g for the Co-EDTA through the ruminal cannula on the 15th day of each period before the morning meal. Consistent with the protocol for measuring the DM degradation, each cow received the mordanted alfalfa corresponding to the hay included in the diet fed in that particular period of the Latin square. Faecal samples were collected from each cow (grab sample) 12, 24, 30, 36, 42, 48, 72 and 96 h after the markers administration. The faeces were weighed, oven-dried at 60°C and

ground (2-mm screen) for the subsequent chemical analysis. The faeces preparation for Cr and Co determination was made according to Murthy *et al.* (1971), by wet-ashing of the samples and the analytic determination of the two elements was then carried out with an atomic absorption spectrophotometer. The dynamics of the ruminal rate of passage of the 2 markers was described by fitting their concentration in the faecal samples with the 2-compartment model proposed by Grovum and Williams (1973). The fitting procedure used DUD within PROC NLIN of SAS (1989).

Rumen fluid parameters

Individual rumen fluid samples were aspirated by a vacuum pump through the rumen cannula 4 h after feeding on day 17 of each Latin square period. After filtration, pH was measured and then a part of the sample was frozen for the subsequent volatile fatty acids (VFA) analysis, while a 15 ml fraction was added with 3 ml of metaphosphoric acid 25% for the N-NH₃ determination.

In vivo digestibility

The digestibility of the diets was calculated with the direct in vivo method Ingesta-Excreta. Total faecal collection was carried out from day 15 to day 19 of each period by taking into account the samples used for rate of passage determination. The faeces excreted by each cow during 24 h were weighed, homogenised with a mechanical mixer and sampled (5%). These samples along with daily samples of the diets and orts were oven-dried at 60°C until constant weight and then kept for the following chemical analysis.

Chemical analysis

All samples of alfalfa hays, diets, orts and faeces collected during the experiment were analyzed for DM, crude protein, ash, and ether extract in accordance with AOAC (1990). The fibrous content of the same samples was determined with the methods proposed by Van Soest *et al.* (1991). The VFA content of the rumen fluid samples collected during the study was determined through gas chromatography according to the procedure suggested by Hamada *et al.* (1968). The ammonia nitrogen content of the same samples was mea-

sured with a specific electrode. Milk samples were analysed for fat and protein content composition by Milko-scan (Foss Electric, Hillorød, Denmark) according to the FIL-IDF standards. The non-protein nitrogen content was measured with the method proposed by Resmini *et al.* (1990).

Statistical analysis

The experimental data were analysed by ANOVA using the GLM procedure of SAS (1989). According to the Latin square design, the statistical model considered the effects of period, cow and diet. Sums of squares for diet were partitioned into a set of orthogonal contrasts to test the effects of the stage of maturity (early vs full flowering) and the germplasm (improved variety vs ecotype).

Results and discussion

Alfalfa hays and diet composition

The chemical compositions of the three alfalfa hays are shown in Table 1. As regards the stage of maturity, an earlier cut of the plant significantly improved the hay quality by increasing the crude protein content (21.4 vs 16.5% DM; $P < 0.01$) and lowering the NDF content (42.2 vs 53.5%; $P < 0.01$). It is interesting to note that the advancing forage

maturity was associated with an increase in cellulose and lignin, while the hemicelluloses did not vary significantly their content (Table 1).

The trend of alfalfa to maintain a constant hemicelluloses concentration in relation to the progress of maturity was also reported in previous experiments that analysed the chemical composition of the plant at the different stages of the vegetative cycle (Nordkvist and Aman, 1986; Alhadhrami and Huber, 1992). As the plant becomes more mature, there is an increased cellulose deposition in the secondary wall, associated with an increasing cross-link formation between lignin and hemicelluloses (Jung, 1989). In this way, the structure of the plant becomes more resistant to lodging but it reduces the digestibility of the cell wall components especially of the hemicelluloses which are linked to the lignin (Jung and Lamb, 2003).

The comparison between improved variety and ecotype carried out at the same stage of maturity (Table 1) showed a significant difference only for the lignin content which was higher for the latter (8.6 vs 8.2%; $P < 0.01$). This result is likely a consequence of the "gigantism" of the Vogherese ecotype which requires a coarse structure of the stems to avoid the lodging.

Table 1. Chemical composition of the three alfalfa hays used in the study.

Alfalfa germplasm		Improved variety		Ecotype	Orthogonal contrast		SEM ¹
		Early flowering	Full flowering	Full flowering	Early vs Full flowering	Variety vs Ecotype	
Samples	n.	4	4	4			
Dry matter	%	89.0	89.7	90.1	*	†	0.1
Crude protein	% DM	21.4	16.1	16.8	**	ns	0.5
Ash	"	12.6	11.4	11.2	ns	ns	0.7
Ether extract	"	1.5	1.4	1.5	ns	ns	0.1
Neutral detergent fibre	"	42.2	53.9	53.0	**	ns	1.0
Acid detergent fibre	"	33.3	43.4	44.5	**	ns	0.3
Hemicellulose	"	8.9	10.4	8.5	ns	ns	0.8
Cellulose	"	26.1	34.1	34.9	**	ns	0.5
Acid detergent lignin	"	5.9	8.2	8.6	**	**	<0.1

¹ Standard error of the mean.

** : $P < 0.01$; * : $P < 0.05$; † : $P < 0.10$; ns : $P > 0.10$.

The composition of the experimental diets formulated with the three alfalfa hays is shown in Table 2. It can be noticed that the better quality of hay obtained from the earlier cut made it possible to increase its percentage of inclusion in the diet up to 44% of the total dietary DM, while the two mature alfalfa forages represented only 36% of the total DM of the respective diets. Moreover, the high protein content of the early flowering alfalfa hay (Table 1) resulted in a marked reduction of the protein concentrates included in its diet (Table 2). A similar change in diet composition has been reported by Llamas-Lamas and Combs (1990) who, formulating isonitrogenous diets with alfalfa hays of increasing maturity (crude protein from 26.7 down to 18.7% DM), had necessarily to increase the amount of protein concentrate included in the ration (soybean meal from 0 up to 18.7% DM).

The great similarity in chemical composition observed for the improved variety and the ecotype (Table 1) lead to the formulation of two diets which were substantially equal in the percentage of inclusion of the different feed ingredients (Table 2).

Intake, in situ degradability and rumen passage rate of the diets and rumen fluid parameters

Stage of maturity and type of alfalfa germplasm did not affect the DM intake which ranged around 16 kg/d for all the diets (Table 3). When intake was expressed as percentage of cow's body weight, the calculated value was on average 2.9% and there were no difference among the experimental diets. These values are in close agreement with the predicted intake calculated on the basis of cows body weight, average milk production and days in milk at the beginning of the study.

Table 2. Feed and chemical composition of the experimental diets formulated with alfalfa hay of different germplasm and stage of maturity.

Alfalfa germplasm Stage of maturity	Improved variety		Ecotype	
	Early flowering	Full flowering	Full flowering	
Feed ingredients:				
Alfalfa hay	% DM	43.7	35.9	36.2
Maize silage	"	19.6	19.6	19.7
Maize meal	"	16.4	17.6	17.7
Soybean meal	"	4.7	13.6	11.8
Dry beet pulps	"	13.0	10.6	11.8
Mineral-vitamin mix ¹	"	2.6	2.6	2.6
Chemical composition:				
Dry matter	%	56.3	56.7	55.9
Crude protein	% DM	14.4	14.8	14.7
Ash	"	7.9	7.3	7.2
Ether extract	"	2.8	2.9	2.9
Neutral detergent fibre	"	39.2	39.8	39.1
Acid detergent fibre	"	25.6	26.4	26.2
Nonfibrous carbohydrates	"	35.7	35.2	36.0

¹ Mineral-vitamin mix contained (/kg of DM) 135 g of Ca, 62.5 g of P, 22.5 g of Mg, 17 g of S, 250 g of NaHCO₃, 1,263,000 U of vitamin A, 84,200 U of vitamin D₃, 1580 mg of vitamin E, 10,500 mg of Vitamin PP; 5300 mg of Zn, 2400 mg of Mn, 1600 mg of Fe, 400 mg of Cu, 100 mg Co, 70 mg of I, 15 mg of Se.

The parameters of dietary DM degradability reported in Table 3 show the great similarity in the kinetics of rumen disappearance of the three experimental diets. This is not surprising, since the rations had a similar chemical composition and they were made with the same feed ingredients differing only for the percentage of inclusion of alfalfa hay (Table 2). Under this particular feeding situation, it is likely that any change in dietary DM degradation induced by stage of maturity or type of germplasm of alfalfa hay was compensated by the variation in the percentage of inclusion of the other feed ingredients needed to maintain an equal chemical composition across diets.

The measurement of ruminal rate of passage showed no differences among diets with regards to the solid phase, which averaged 3.6%/h (Table 3). The diet made with the earlier cut of alfalfa had a slower rate of passage of the liquid phase in comparison to those including more mature hays (6.9 vs 7.4%/h; $P < 0.05$). A possible explanation for this result could arise from the lower lignified fibre content of the alfalfa cut at early flowering (Table

1) which, in comparison with the more mature forage, might have promoted a shorter chewing time of the diet with the consequent production of less saliva. The solid and liquid passage rates measured in the present research were much lower than data from other studies using alfalfa forage based diets for dairy cows (Llamas-Lamas and Combs, 1990, 1991). This difference can easily be explained by the productive records of the cows used in the previous researches. Both Llamas-Lamas and Combs studies, in fact, were carried out on Holstein Friesian cows producing more than 33 kg of milk/d with a DM intake always exceeding the 3.5% of the cow's body weight.

The effective degradability values of dietary DM calculated using the rate of passage of the solid phase averaged 62%, once again without any significant effect due either to the stage of maturity or to the germplasm of alfalfa (Table 3). Consistent with the result of the degradation process, also the rumen fluid data were not affected by the same factors (Table 4). Considering the time of the collection in relation to the time of feeding,

Table 3. Dry matter intake, ruminal degradability and passage rate of the experimental diets formulated with alfalfa hay of different germplasm and stage of maturity.

Alfalfa germplasm	Improved variety		Ecotype	Orthogonal contrast		SEM ¹	
	Early flowering	Full flowering	Full flowering	Early vs Full flowering	Variety vs Ecotype		
Intake	kg/d	16.3	15.8	16.0	ns	ns	0.2
	% BW	2.9	2.9	2.8	ns	ns	<0.1
Degradability parameters:							
A ²	%	30.8	31.3	31.6	ns	ns	0.9
Lag phase	h	0.1	0.3	0.3	ns	ns	0.2
B ³	%	53.1	50.9	52.1	ns	ns	1.2
k _B ⁴	%/h	6.1	5.16	5.8	ns	ns	1.0
Rate of passage:							
Solid phase	"	3.75	3.35	3.75	ns	ns	0.12
Liquid phase	"	6.89	7.46	7.39	*	ns	<.01
Effective degradability	%	63.5	61.6	61.9	ns	ns	1.1

¹ Standard error of the mean.

² A = readily degradable fraction

³ B = fraction degradable at measurable rate.

⁴ k_B = degradation rate

*: $P < 0.05$; ns: not significant ($P > 0.10$).

Table 4. Rumen fluid parameters of dairy cows fed the experimental diets formulated with alfalfa hay of different germplasm and stage of maturity.

Alfalfa germplasm	Improved variety		Ecotype	Orthogonal contrast		SEM ¹	
	Early flowering	Full flowering	Full flowering	Early vs Full flowering	Variety vs Ecotype		
pH	6.3	6.2	6.2	ns	ns	0.1	
Volatile fatty acids:							
Total	mmol/l	100.1	103.8	104.4	ns	ns	8.5
Acetic - C2	%	65.2	65.9	65.1	ns	ns	1.7
Propionic - C3	"	21.0	20.0	20.5	ns	ns	1.5
Butyric	"	11.2	11.4	11.3	ns	ns	0.2
Valeric	"	2.7	2.8	2.7	ns	ns	0.1
C2/C3		3.1	3.3	3.2	ns	ns	0.3
N-NH ₃	mg/l	85.2	92.0	84.4	ns	ns	13.3

¹ Standard error of the mean.
ns: not significant ($P > 0.10$).

rumen fluid pH and VFA profile must be considered suitable for a lactating cow. In the case of the N-NH₃ concentration the experimental values observed for all the diets were far above the 50 mg/l which according to Satter and Slyter (1974) should allow the more efficient microbial growth. This result is strongly related to the main source of proteins used in the formulation of the experimental diets since both soybean meal and alfalfa hay must be considered readily degradable in the rumen (NRC, 2001).

In vivo digestibility of the diets

The digestibility coefficients of several chemical constituents of the experimental diets are shown in Table 5. The DM digestibility of the diets averaged 66% without any significant differences in relation to alfalfa cultivar or vegetative stage. The lack of significant effects due to the type of hay was observed also for the different chemical constituents considered (Table 5). In spite of the lack of statistical significance among diets, it is particularly interesting to note within each diet the change in the contribution of the different fibrous fractions to the digestible NDF which was likely caused by the type of alfalfa hay. In the diet with the early flowering alfalfa, the coefficient of hemicelluloses digestibility was higher than that

of cellulose. An opposite result was instead observed for the diets including more mature alfalfa forage (Table 5). This observation seems to confirm the depressive effect of the advancing maturity on hemicelluloses digestibility in alfalfa, due to the progressive development of the links between hemicelluloses, uronic acids and the phenolic acids of lignin (Jung, 1989). Sullivan (1966), in this respect, found a negative correlation ($r = -0.83$) between lignin content and hemicelluloses digestibility. However, more recently a negative correlation ($r = -0.80$) between lignin content and fibre digestibility was also reported by Cherney *et al.* (1993) and this seems to suggest that lignin does not have a detrimental effect only on hemicelluloses digestibility. This hypothesis was supported by Fukushima *et al.* (1991), who found that lignin has an inhibitory effect on alfalfa cellulose digestibility likely due either to physical encrustation of lignin or to its chemical binding with this fibrous fraction.

In the present study, the relation between lignin content and fibre digestibility was also investigated at same stage of maturity in the comparison between the diet formulated using the different germplasms (Table 5). The results showed the negative effect of the ecotype, richer in lignin, only as a trend, since its hemicelluloses and cellu-

lose digestibility did not reach the minimum threshold of the statistical significance.

Crovetto *et al.* (1993) found an increased hemicelluloses and cellulose digestibility for Boreal in comparison with an other local ecotype of the Po Valley (Giant cremonese) feeding the two hays ad libitum as the sole feedstuff to adult rams. Based on our results, the effect of the germplasm on the digestibility of the fibrous fractions appears less evident when alfalfa is part of a total mixed ration.

Milk yield and composition

The milk production did not show significant changes due to maturity or to the cultivar of alfalfa (Table 6). With regards to milk composition, a higher fat content was observed in the milk produced feeding the diet with the ecotype in comparison to the improved variety (4.45 vs 4.02; $P < 0.10$). There is not a clear biological explanation for this result since DM intake, its degradation process and the passage rate (Table 3), the conditions of the rumen environment (Table 4) and the total tract digestibility (Table 5) were similar between the two diets.

A significant effect of alfalfa maturity was observed for the milk protein which was lowered when the cows received the diet with the early cut hay (Table 6). This decrease was not due to a reduction of the milk non-protein nitrogen and therefore regarded the true protein. It must be

pointed out that the early flowering diet had the highest inclusion of alfalfa hay which replaced soybean meal in order to equalize the protein content across diets (Table 2). Alfalfa protein is more degradable than soybean meal in the rumen (NRC, 2001) and therefore an increasing inclusion of the forage in the ration lowered the dietary protein that was able to by-pass the fore stomachs. According to Llamas-Lamas and Combs (1991), the decreased milk protein synthesis observed in high producing dairy cows fed a considerable amount of alfalfa forages in their ration could arise from the lower by-pass protein quantity reaching the duodenum. In our study, the modest level of milk production and the similar value of ammonia nitrogen measured in the rumen fluid 4 h after feed delivery (Table 4) do not seem to fully support this hypothesis. However, several studies suggest the need to use high by-pass protein sources in alfalfa-based diets (Broderick *et al.*, 1990; Wattiaux *et al.*, 1994), underlining that the positive productive response to their use can be balanced only by an extremely high DM intake. This was not the case of the present research.

Conclusions

The study confirmed the positive improvement in the chemical composition of alfalfa hay resulting from the cut of the plant at an earlier stage of

Table 5. *In vivo* digestibility of the chemical constituents of the experimental diets formulated with alfalfa hay of different germplasm and stage of maturity.

Alfalfa germplasm	Stage of maturity	Improved variety		Ecotype	Orthogonal contrast		SEM ¹
		Early flowering	Full flowering	Full flowering	Early vs Full flowering	Variety vs Ecotype	
Dry matter	%	67.8	66.6	65.9	ns	ns	1.6
Crude protein	"	66.0	64.2	66.0	ns	ns	1.0
Ether extract	"	74.3	72.7	76.9	ns	ns	0.9
Neutral detergent fibre	"	49.2	51.1	47.2	ns	ns	3.7
Acid detergent fibre	"	40.5	47.8	44.3	ns	ns	5.9
Hemicelluloses	"	65.4	57.4	52.9	ns	ns	7.0
Cellulose	"	50.6	59.6	56.3	ns	ns	5.5

¹ Standard error of the mean.

ns: not significant ($P > 0.10$).

Table 6. Milk yield and composition of dairy cows fed the experimental diets formulated with alfalfa hay of different germplasm and stage of maturity.

Alfalfa germplasm		Improved variety		Ecotype	Orthogonal contrast		SEM ¹
		Early flowering	Full flowering	Full flowering	Early vs Full flowering	Variety vs Ecotype	
Milk yield	kg/d	19.7	19.1	20.0	ns	ns	0.3
Milk fat	%	4.29	4.02	4.45	ns	†	0.09
	g/d	848	770	902	ns	†	19
Milk protein	%	3.39	3.49	3.50	**	ns	0.02
	g/d	667	669	699	ns	ns	10
Milk non-protein nitrogen	%	0.18	0.18	0.18	ns	ns	0.04

¹ Standard error of the mean.

***P*<0.01; †*P*<0.10; ns: not significant (*P*>0.10).

maturity. Compared to more mature legume forage, this hay can increase its percentage of inclusion in diets for dairy cattle, due to the high protein and low NDF content. However, since alfalfa protein is known for high rumen degradability, in the managing of this type of alfalfa hay in dairy feeding, some concern must be addressed to the diet formulation in order to prevent the possible milk protein depression caused by an excess of degradable protein. To overcome this problem, diets with a great amount of early cut alfalfa should: 1) include readily available sources of carbohydrates in order to match the high degradable protein of alfalfa with the energy needed to promote an efficient microbial growth; 2) consider as protein supplements feedstuffs with a high rumen by-pass in order to increase the amount of protein reaching the duodenum.

The comparison between an improved variety and an ecotype of alfalfa, carried out at the same stage of maturity has not shown significant differences from the nutritional point of view. The higher lignin content of the ecotype had no detrimental effects on the digestive process of the forage when part of a total mixed ration. Therefore, an ultimate decision about the choice of one of these germplasms should be based on their field yield.

The results of the digestibility trial confirmed the negative correlation between the lignin content and the digestibility of the main fibre frac-

tions of alfalfa. Therefore, in spite of the important role played by alfalfa lignin as ruminal buffer, this cell wall constituent and its links with the hemicelluloses and cellulose remain the greatest obstacles for the improvement of the nutritional properties of alfalfa forage to be used in dairy cow rations.

REFERENCES

- ALHADHRAMI, G., HUBER, J.T., 1992. Effects of alfalfa hay of varying fiber fed at 35 or 50% of diet on lactation and nutrient utilization by dairy cows. *J. Dairy Sci.* 75:3091-3099.
- ANDRIGHETTO, I., COZZI, G., MAGNI, G., HARTMAN, B., HINDS, M., SAPIENZA, D., 1995. Comparison of in situ degradation kinetics of Lucerne germplasm by ANOVA of non-linear models. *Anim. Feed Sci. Technol.* 54:287-299.
- AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Washington, DC, USA.
- BEAUCHEMIN, K.A., YANG, W.Z., RODE, L.M., 2003. Effects of particle size of alfalfa-based dairy cow diets on chewing activity, ruminal fermentation, and milk production. *J. Dairy Sci.* 86:630-643.
- BRODERICK, G.A., BRADFORD RICKER, D., SPENCE DRIVER, L., 1990. Expeller soy bean meal and corn by-products versus solvent soybean meal for lactating dairy cows fed alfalfa silage as sole forage. *J. Dairy Sci.* 73:453-462.
- CHERNEY, D.J.R., CHERNEY, J.H., LUCEY, R.F., 1993. In vitro digestion kinetics and quality of perennial grasses as influenced by forage maturity. *J. Dairy*

- Sci. 76:790-797.
- COZZI, G., BERZAGHI, P., GOTTARDO, F., ANDRIGHETTO, I., 1999. Risposta produttiva di bovine frisone in fase iniziale di lattazione alla somministrazione di due diverse fonti di proteina indegradabile. *Zoot. Nutr. Anim.* 25:207-217.
- FUKUSHIMA, R.S., DEHORITY, B.A., LOERCH, S.C., 1991. Modification of a colorimetric analysis for lignin and its use in studying the inhibitory effects of lignin on forage digestion by ruminal microorganisms. *J. Anim. Sci.* 69:295-304.
- GROVUM, W.L., WILLIAMS, V.J., 1973. Rate of passage of digesta in sheep. 4. Passage of marker through the alimentary tract and biological relevance of rate-constants derived from the changes in concentration of marker in faeces. *Br. J. Nutr.* 30:313-329.
- HAMADA, T., OMORI, S., KAMEOKA, K., HORII, S., MORIMOTO, H., 1968. Direct determination of rumen volatile fatty acids by gas chromatography. *J. Dairy Sci.* 51: 228-229.
- HAYS, D.B., SKINNER, D.Z., 2001. Development of an expressed sequence tag (EST) library for *Medicago sativa*. *Plant Sci.* 161:517-526.
- HERMANN, M.L., RUSSELL, J.R., BARNHART, S.K., 2002. Evaluation of hay-type and grazing-tolerant alfalfa cultivars in season-long or complementary rotational stocking systems for beef cows. *J. Anim. Sci.* 80:768-779.
- JUNG, H.G., 1989. Forage lignins and their effects on fiber digestibility. *Agron. J.* 81:33-38.
- JUNG, H.G., LAMB, J.F.S., 2003. Identification of lucerne stem cell wall traits related to in vitro neutral detergent fibre digestibility. *Anim. Feed Sci. Technol.* 110:17-29.
- LLAMAS-LAMAS, G., COMBS, D.K., 1990. Effect of alfalfa maturity on fiber utilization by high producing dairy cows. *J. Dairy Sci.* 73:1069-1080.
- LLAMAS-LAMAS, G., COMBS, D.K., 1991. Effect of forage to concentrate ratio and intake level on utilization of early vegetative alfalfa silage by dairy cows. *J. Dairy Sci.* 74:526-536.
- MERTENS, D.R., LOFTEN, J.R., 1980. The effects of starch on forage fiber digestion kinetics in vitro. *J. Dairy Sci.* 63:1437-1446.
- MINISTRY OF AGRICULTURAL AND FORESTRY POLICIES, 2004. Registri nazionali delle varietà vegetali. Home page address: <http://www.politicheagricole.it/mipa/banchedati/mnuRVV.htm>
- NORDKVIST, E., AMAN, P., 1986. Changes during growth in anatomical and chemical composition on in vivo-degradability of lucerne. *J. Sci. Food Agric.* 37:1-7.
- NRC 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press, Washington, DC, USA.
- ØRSKOV, E.R., HOVELL, F.D., MOULD, F., 1980. The use of nylon bag technique for the evaluation of feed-stuffs. *Trop. Anim. Prod.* 5:195-213.
- Ramanzin, M., Bittante, G., Bailoni, L., 1991. Evaluation of different Chromium-mordanted wheat straws for passage rate studies. *J. Dairy Sci.* 74:2989-2996.
- RESMINI, P., TRIPICIANO, C., RAMPILLI, M., LODI, R., 1985. Alcuni aspetti del controllo di qualità del latte al consumo. *Riv. Soc. Ital. Sci. Aliment.* 3:187-195.
- RUPPERT, L.D., DRACKLEY, J.K., BREMMER, D.R., CLARK, J.H., 2003. Effects of tallow in diets based on corn silage or alfalfa silage on digestion and nutrient use by lactating dairy cows. *J. Dairy Sci.* 86:593-609.
- SAS, 1989. User's Guide: Statistics, Version 6. Sas Institute Inc., Cary, NC, USA.
- SATTER, L.D., SLYTER, L.L., 1974. Effect of ammonia concentration of rumen microbial protein production in vitro. *Br. J. Nutr.* 32:199-208.
- SULLIVAN, J.T., 1966. Studies of the hemicelluloses of forage plants. *J. Anim. Sci.* 25:83-86.
- VAN SOEST, P.J., ROBERTSON, J.B., LEWIS, B.A., 1991. Method for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
- WATTIAUX, M.A., COMBS, D.K., SHAVER, R.D., 1994. Lactational response to ruminally undegradable protein by dairy cows fed diets based on alfalfa silage. *J. Dairy Sci.* 77:1604-1617.
- ZILLOTTO, U., ANDRIGHETTO, I., COZZI, G., SCOTTON, M., 1993. Quali-quantitative aspects of lucerne (*Medicago sativa* L.) production in relation to cultivar and utilization rate. In: P. Rotili and L. Zannone (eds.) *The future of Lucerne Biotechnology Breeding and Variety Constitution*. Istituto Sperimentale per le Colture Foraggere ed., Lodi, Italy, pp 130-138.